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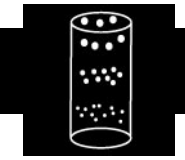

Field Flow Fractionation

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Introduction

Two subtechniques of the field-flow fractionation (FFF) family are used to separate polymers with high resolution on an analytical scale; these are thermal FFF (ThFFF) and flow FFF (FlFFF). For lipophilic polymers, ThFFF excels in the analysis of high-molecular-weight-polymers ($M > 10^6\,\mathrm{g\,mol^{-1}}$) and gel-containing polymers. ThFFF can also separate polymer blends and copolymers according to chemical composition. For hydrophilic polymers, FlFFF compares well with size-exclusion chromatography (SEC) for the analysis of polymers with $M > 10^3\,\mathrm{g\,mol^{-1}}$, and like ThFFF, excels when $M > 10^6\,\mathrm{g\,mol^{-1}}$. By varying factors that control retention, each FFF application can be optimized, and programming such factors allows highly polydisperse samples to be analysed with unparalleled precision in a single run. FFF channels are more expensive than SEC columns, but with proper maintenance, channel lifetimes are virtually unlimited.

FFF, like liquid chromatography, relies on the differential migration of dissolved or suspended materials as they are flushed through a conduit. Unlike chromatography, however, the FFF separation relies on interactions of the analyte with an applied field rather than a stationary phase. As a result, the FFF separation occurs in a single phase (see **Figure 1**) with minimal exposure to surfaces, and the flowing liquid has a laminar profile. These features make for a gentle separation, so that fragile molecules and molecular complexes can be characterized with little disruption.

FFF instrumentation (**Figure 2**) is similar to that for chromatography, and consists of a pump to drive the carrier liquid, an injection port, the separation channel, and a detector to monitor the channel effluent.

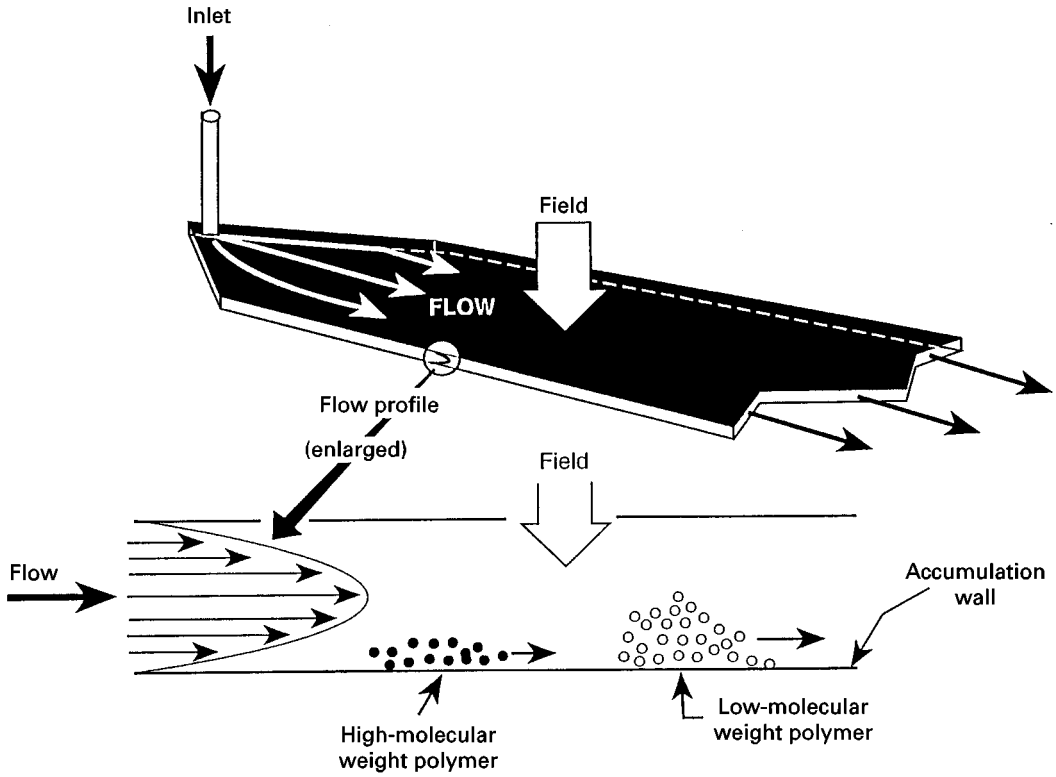

Figure 1 Illustration of the separation mechanism in FFF. The field compresses larger material into a thinner layer against the accumulation wall, where they move more slowly.

A computer is used to control the applied field and to store the detector signal. Samples are injected with a microsyringe, either directly or via an injection valve.

One of the greatest strengths of FFF is its ability to directly measure physicochemical parameters on analyte components using well-defined models of retention. In FlFFF, for example, the diffusion coefficient (D) can be calculated directly from a component's retention time. From D, the hydrodynamic size can be calculated, and if the intrinsic viscosity is measured independently, the molecular weight can be determined. Molecular weight can also be obtained directly from retention measurements through calibration standards. In ThFFF, D values can also be calculated from measured retention times once an additional parameter for each polymer type is obtained, as discussed below. An additional advantage

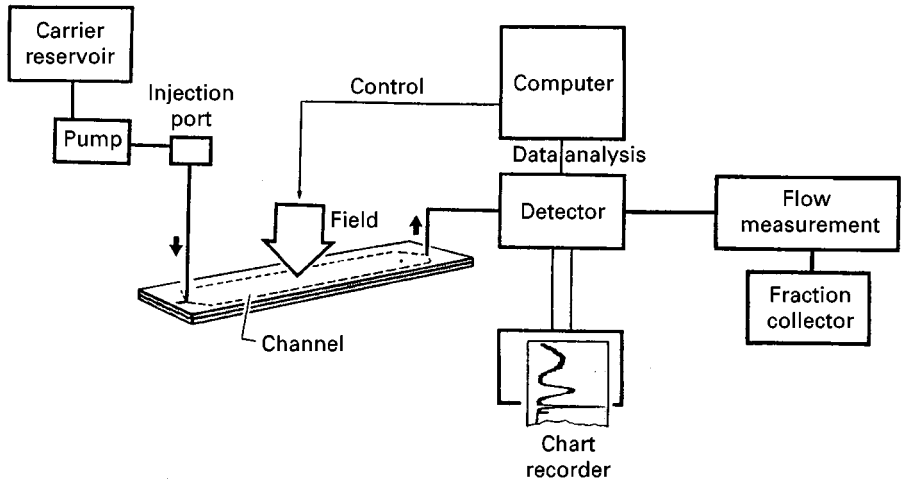

Figure 2 Schematic diagram of the FFF instrument.

of ThFFF is that band broadening is well defined, allowing for the determination of highly precise molecular-weight determinations.

Besides its placid nature and theoretical tractability, another attractive feature of FFF is its applicability to a wide variety of materials and situations. For example, FlFFF has been used to separate materials ranging in size from 10^3 to 10^{18} g mol^{-1}. However, flexibility comes with a price, and the user must understand the separation mechanism in order to apply FFF to new and different samples with efficiency. Outlined below are the more common applications of both ThFFF and FlFFF for polymer analysis with comparisons, when appropriate, to SEC.

Principles and Theory of Retention

The FFF channel has the shape of a ribbon (Figure 1), with a length of typically 30–50 cm, a breadth of 1–3 cm, and a typical thickness of 0.05–0.25 mm. A stream of carrier liquid is introduced at one end of the channel and exits at the other end. Since the channel has a high aspect ratio, the flow of carrier liquid is laminar, with a parabolic velocity profile across the thin dimension. A field is applied across the thin dimension, and a mixture to be separated is injected at the inlet end of the channel. As the mixture is transported by carrier liquid to the outlet, interactions with the field compress the sample against one wall, where slower streamlines exist. The concentration of sample at the accumulation wall is opposed by diffusion, and the result is a sample cloud with a concentration that decreases exponentially with distance from the wall. Components that interact differently with the field will form zones of different thickness at the accumulation wall. The dependence of zone thickness l on the force F of the interaction with the field is:

$$l = kT/F \qquad [1]$$

where k is Boltzmann's constant and T is temperature. The thickness of the zone determines the extent to which its migration through the channel is reduced.

The extent to which an analyte is retained in FFF can be specified, as in chromatography, by its retention ratio R:

$$R = t^\circ/t_r = V^\circ/V_r \qquad [2]$$

where t_r and V_r are the time and volume of carrier liquid, respectively, required to flush a component through the channel; the void time t° and void volume V° are the analogous parameters for a component that does not interact with the field. The dependence of retention ratio R on zone thickness l is:

$$R = 6\frac{l}{w}\left[\coth\left(\frac{1}{2l/w}\right) - 2\frac{l}{w}\right] \qquad [3]$$

The ratio l/w is given the symbol λ, and is referred to as the retention parameter, since it alone describes the relative migration of a component zone. As $\lambda \to 0$, $R \to 0$, and the analyte does not move through the channel. As $\lambda \to \infty$, $R \to 1$, and the analyte moves at the average velocity of the carrier liquid. As λ is reduced, the bracketed term in eqn [3] approaches unity, so that for many applications the relationship between R and λ is described by the following simple equation:

$$R = 6\lambda \qquad [4]$$

The retention ratio of an eluting component can be determined experimentally through eqn [2] and translated into a λ value using eqn [3]. Values of λ, in turn, can be related to physicochemical properties of the analyte, as discussed below.

The properties that govern F (or λ) vary with the nature of the applied field, i.e. with the FFF subtechnique. In all sub-techniques, however, retention varies directly with the magnitude of the applied field. This relationship facilitates tuning the field in order to optimize each application, so that routine analyses can be performed with maximum efficiency. For highly polydisperse samples, the magnitude of the field can even be programmed in order to reduce the separation time of such samples. Field programming is analogous to temperature programming in gas chromatography and gradient elution in liquid chromatography. **Figure 3** illustrates the ThFFF separation of seven polymer standards ranging in M from 9000 to 5.5×10^6 g mol^{-1} in a single run.

Thermal FFF

In ThFFF, the applied field is a temperature gradient formed by heating and cooling, respectively, the two walls that define the thin dimension of the channel. A schematic of the ThFFF channel is illustrated in **Figure 4**. When placed in a temperature gradient, polymers migrate toward the lower temperature. This effect, which in known as thermal diffusion, governs the retention parameter (λ) in the following way:

$$\lambda_{Th} = \frac{D}{D_T \Delta T} \qquad [5]$$

Here D_T is the coefficient of thermal diffusion, which relates mass flux to a temperature gradient, and ΔT is

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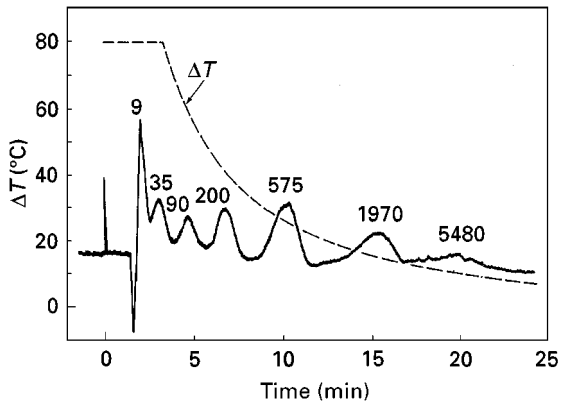

Figure 3 Separation of a seven-component mixture by ThFFF with field programming. Values above peaks are molecular weights expressed as $\times 10^3$ g mol^{-1}. Reprinted with permission from J. C. Giddings, V. Kumar, P. S. Williams and M. N. Myers (1990). In: Craver D and Provder T (eds) *Polymer Characterization by Interdisciplinary Methods, ACS Advances in Chemistry Series No. 227,* C. Washington, D.C.: ACS Publications.

the temperature drop across the channel. Equation [5] is actually an approximation because of an assumption that the temperature gradient is constant; this is not strictly true because solvent thermal conductivity changes with temperature across the channel. In fact, eqn [3] is also an approximation for ThFFF because of the temperature dependence of the solvent viscosity, which leads to a skewed velocity profile. Various approaches have been used to refine eqns [3] and [5] in order to account for such temperature effects (see Martin, 1998), but for routine polymer analysis such refinements are not necessary.

The dependence of λ_{Th} on D/D_T means that neither D nor D_T can be computed by itself, only the ratio D/D_T. Fortunately, D_T is independent of molecular weight and branching configuration for a given polymer–solvent system, at least for random-coil homopolymers. As a result, λ_{Th} is a linear function of D for a given system when ΔT is held constant. Thus, once D_T is determined for a given system, values of D can be calculated directly from measurements of λ_{Th} in that system.

Since D_T is independent of molecular weight M, the separation of polymers by ThFFF is rooted, like SEC, in the dependence of D on M; that dependence is given by the following expression:

$$D = \frac{kT}{6\pi\eta_0}\left(\frac{10\pi N_A}{3M[\eta]}\right)^{1/3} \qquad [6]$$

where η_0 is the viscosity of the solvent, N_A is Avagadro's number, and $[\eta]$ is the intrinsic viscosity of the polymer. The relationship defined by eqn [6] forms the basis for universal calibration in SEC, and is applicable to ThFFF provided D_T is known for each polymer–solvent system to which the universal calibration is applied.

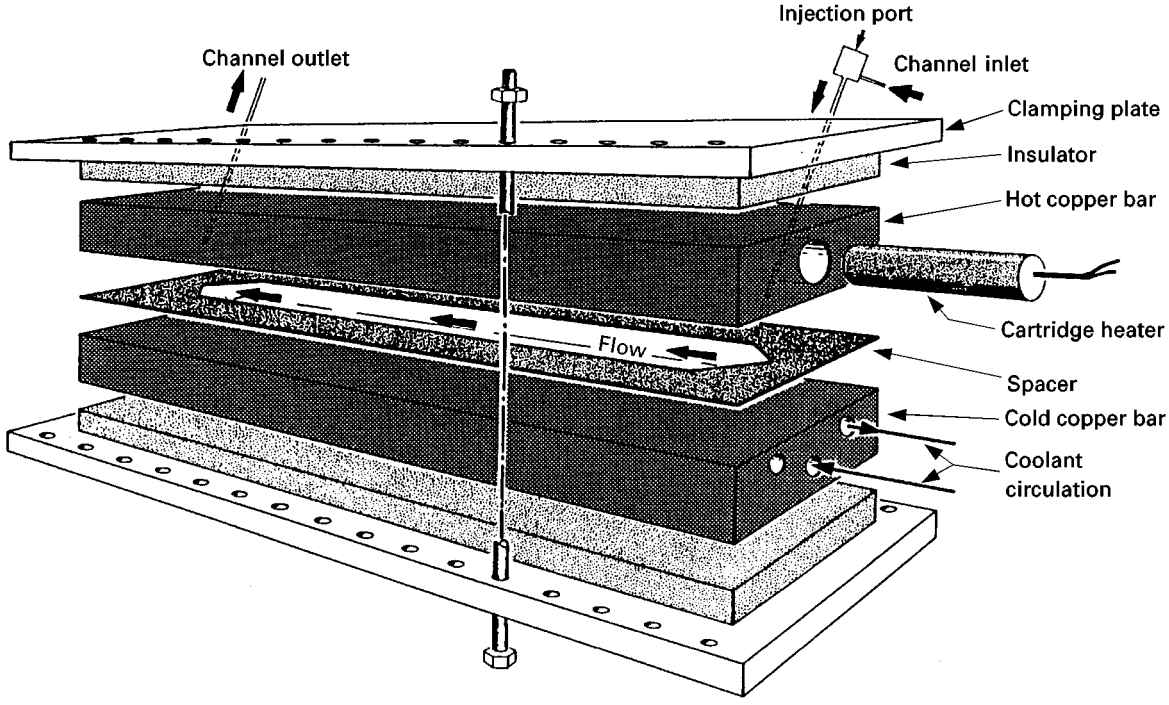

Figure 4 Basic design of the ThFFF channel, which is formed by a spacer sandwiched between two nickel-coated copper bars. One of the bars is heated while the other is cooled.

Flow FFF

In FlFFF, the applied field is a flow of carrier liquid across the thin dimension of the channel. This cross-flow is made possible by constructing one or both channel walls with a fritted material that is permeable to the carrier liquid (**Figure 5**). As a result, the flowing liquid has two perpendicular vectors. The axial-flow vector lies along the length of the channel, has a parabolic velocity profile across the thin dimension, and carries sample through the channel as in other FFF subtechniques. The cross-flow vector is directed across the channel, has a relatively flat velocity profile, and serves as the applied field by physically transporting material to the accumulation wall. A semipermeable membrane placed against the accumulation wall prevents analyte from penetrating the wall, while allowing the carrier liquid to pass through.

FlFFF employs one of two channel designs.

- In the symmetric design (SyFlFFF), both the accumulation wall and the (opposite) depletion wall are porous, and the axial flow and cross flow are controlled independently with separate pumps.
- In the asymmetric design (AsFlFFF), the depletion wall is replaced with a glass plate so that a single inlet stream serves as the source for both axial flow and cross flow.

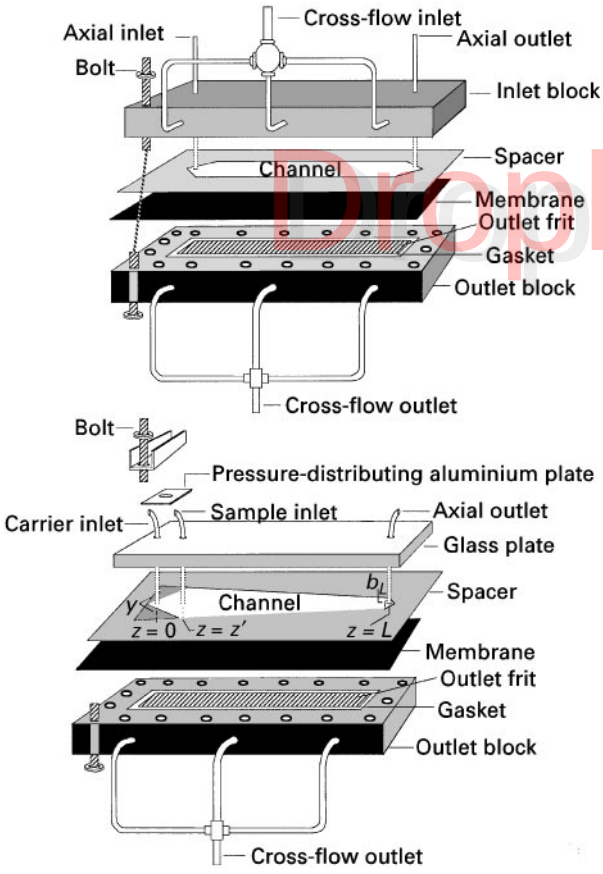

Figure 5 Basic design of the flow FFF channel. In the symmetrical channel (top), the channel spacer is sandwiched between two frits, which allow carrier liquid to flow across the thin dimension of the channel. In the asymmetrical channel (bottom), a solid plate replaces the upper frit, which is transparent in order to see inside the channel during operation.

The relative magnitudes of the two flow vectors are controlled by adjusting the relative amount of back-pressure applied at the axial outlet versus the cross-flow outlet. In contrast to the symmetric design, the axial velocity diminishes along the length of an asymmetric channel as fluid is lost through the accumulation wall. To compensate for this effect, the width of the asymmetric channel is tapered from inlet to outlet. However, except for a specific ratio of cross-to-axial flow rates, the axial velocity will still vary along the length of the channel. Therefore, for AsFlFFF, eqn [3] is not valid and must be replaced with:

$$R = \frac{t^\circ}{t_r} = \frac{6}{w}\frac{\int_0^w e^{(-x/l)B(x)}x\,dx - \frac{1}{w}\int_0^w e^{(-x/l)B(x)}x^2 dx}{\int_0^w e^{(-x/l)B(x)}\,dx} \quad [7a]$$

Here x is the distance from the accumulation wall:

$$B(x) = 1 - \frac{x^2}{w^2} + \frac{x^3}{2w^3} \quad [7b]$$

and:

$$t^\circ = \frac{V^\circ}{V_C}\ln\left(1 + \frac{V_C}{V_{out}}\left\{1 - \frac{w\left[b_o z' - \frac{(b_o - b_L)(z')^2}{2L} - y\right]}{V^\circ}\right\}\right) \quad [7c]$$

where V_C and V_{out} are the volumetric rates of flow thorough the cross-flow and axial-flow outlets, respectively. Parameters b_o and b_L are the breadths of the channel at the sample inlet and outlet, respectively, z' is the distance between the carrier inlet and the focusing position (discussed below), and y is the area reduction of the accumulation wall due to the tapered inlet (see **Figure 5**).

Asymmetric channels have two primary advantages: (1) they are less costly, and (2) the inside of the channel can be seen through the glass plate. By observing the motion of an injected dye, flow irregularities caused by a poorly sealed channel are easily visualized. On the other hand, the advantages of the

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symmetric design are: (1) the axial flow and cross flow can be controlled independently, and (2) the equations relating R to λ are simpler.

In both FlFFF channel designs, the cross flow pushes all components with the same velocity (U) toward the accumulation wall. As a result, only the opposing motion of diffusion governs retention:

$$\lambda_F = \frac{D}{Uw} = \frac{DV^\circ}{V_C w^2} \qquad [8]$$

Like ThFFF, the well-established inverse dependence of λ on field strength imparts flexibility and allows field programming, so that the most efficient possible method can be developed for each application.

Application to Polymer

Within the FFF family, the choice between thermal and flow FFF is a simple one for polymer analysis. In general, FlFFF is used for hydrophilic polymers, while ThFFF is best suited to lipophilic polymers. In either case, an advantage that FFF has over SEC is its greater peak capacity. In principle, V_r is unlimited in FFF, although 20 channel volumes represent a practical limit. In SEC, V_r is limited at the high end by the permeation volume (equal to one column volume), and at the low end by the exclusion volume.

Lipophilic Polymers

For lipophilic polymers with $M < 10^4\ \mathrm{g\ mol^{-1}}$, ThFFF suffers from a lack of resolution, therefore SEC is almost mandatory, and certainly preferred. However, above $10^4\ \mathrm{g\ mol^{-1}}$, the resolving power of ThFFF increases rapidly, and exceeds that of SEC for $M > 10^5\ \mathrm{g\ mol^{-1}}$. For ultra-high molecular-weight polymers ($M > 10^6$), SEC becomes increasingly limited by shear-induced fragmentation of the chains as they travel through the packed bed under high pressure, and ThFFF is clearly superior.

Between 10^4 and $10^6\ \mathrm{g\ mol^{-1}}$, neither SEC nor ThFFF has an overwhelming advantage for the analysis of many polymers. In general, ThFFF is more difficult to implement than SEC because there are more factors under the control of the user that influence retention. While this adds flexibility, only by understanding the separation mechanism and governing equations can one avoid certain pitfalls in choosing the proper parameters for each application.

For analysing certain types of lipophilic polymers, ThFFF has some rather unique advantages. The absence of shear forces, which make ThFFF especially suited to ultra-high molecular-weight polymers, was mentioned above. Using re-injection techniques, and the absolute measurement of M by light scattering, the integrity of ThFFF analyses on high molecular-weight polymers that degrade in SEC columns has been clearly demonstrated. The open ThFFF channel is also amenable to gel-containing polymers. Since sample filtration is not required, microgels are not lost in the analysis, and an estimate of the gel content can even be obtained. ThFFF is also well suited to polyolefins, which are difficult to separate by SEC because high temperatures ($>130^\circ\mathrm{C}$) are required for their dissolution. At these temperatures, column packings used in SEC tend to degrade at an elevated rate, while the ThFFF channel is more robust.

Although the thermal diffusion coefficient D_T is independent of molecular weight, it varies with polymer composition. As a result, ThFFF can resolve polymer components that differ chemically even when their diffusion coefficients (or hydrodynamic volumes) are identical. This is in contrast to SEC, where components with similar diffusion coefficients cannot be separated. The dependence of retention on polymer composition can be used to separate copolymers according to composition, and when the dependence of D_T on copolymer composition is known, the chemical composition can be calculated from retention data. Such is the case for random (statistical) copolymers, where D_T is a weighted-average of the D_T values of the homopolymer constituents, with the weighting factors being the mole-fractions of each component in the copolymer. Thus, by measuring the retention of a copolymer of unknown composition, its D/D_T value can be calculated using eqns [3] and [5]. With an independent measure of D, a value for D_T can be calculated, and from D_T the copolymer composition. For block copolymers, a linear dependence of D_T on composition requires the polymers to be dissolved in a non-selective solvent, which is a solvent that is equally good for all copolymer components. Unfortunately, with highly branched block copolymers, even a non-selective solvent will fail to yield a linear dependence.

ThFFF is incapable of resolving the components of certain polymer mixtures. For example, when the composition of a polydisperse copolymer changes with molecular weight, two components that differ in both molecular weight and composition may have the same D/D_T ratio, even though their individual values of D and D_T differ. Such components will co-elute, in which case the combination of SEC and ThFFF is extremely powerful. Components can first be separated according to differences in D using SEC, then fractions from the SEC column, which are homogeneous in D, can be further separated according to D_T by ThFFF. **Figure 6** illustrates such a combination applied to a polymer–copolymer mixture that neither

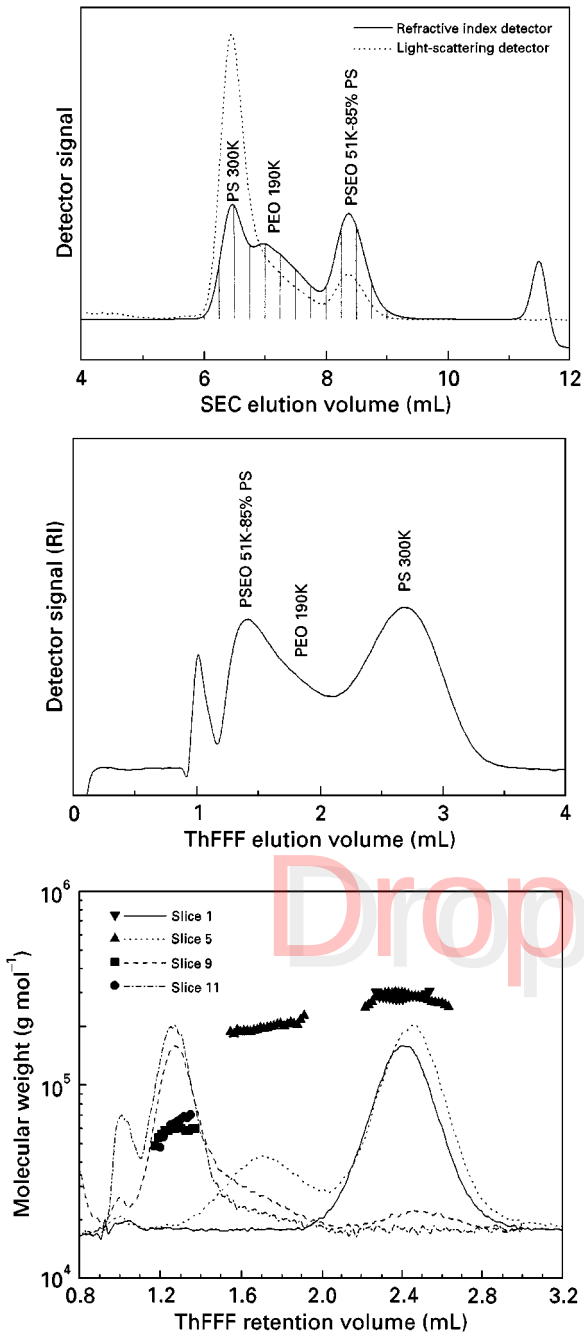

Figure 6 Cross-fractionation of a three-component polymer mixture by SEC and ThFFF. The mixture could not be sufficiently resolved for characterization by either SEC (top) or ThFFF (middle) alone. Cross-fractionation of SEC elution slices (bottom) provided enough resolution to determine the molar mass of each component with a multi-angle light-scattering detector (Dawn DSP, Wyatt Technology, Santa Barbara, CA). The composition of the components were determined from D and D_T values calculated from SEC and ThFFF retention volumes, respectively.

SEC nor ThFFF alone can separate. By cross-fractioning the mixture, the three components were sufficiently resolved to determine both the molecular weight and composition of each component.

Highly precise information on the polydispersity of lipophilic polymers can be obtained with ThFFF because column dispersion is well modelled, and its effect on the elution profile can therefore be removed. For example, plots of plate height H versus flow rate are linear. Such plots can be extrapolated to zero flow rate to yield an intercept term from which the sample polydispersity can be calculated. This method is used to obtain highly precise measurements of the polydispersity ($\mu < 1.005$) of polymers prepared by anionic polymerization. By comparison, the precision of SEC for such measurements is reduced by an order of magnitude because of uncertainties in the contribution of column dispersion to plate height. For a more detailed analysis, a well-defined band-broadening function can be mathematically removed from the elution profile to obtain highly precise molecular-weight distributions. With more polydisperse polymers ($\mu < 1.005$), column dispersion is nearly negligible in ThFFF when typical flow rates are used, so that elution profiles can be converted directly into accurate molecular-weight distributions.

Hydrophilic Polymers

For analysing hydrophilic polymers, FlFFF shares many of the advantages and limitations of ThFFF when compared to SEC. A notable difference is that FlFFF can be extended to lower molecular weights (10^3 g mol^{-1}). Another difference is that the effects of column dispersion cannot be completely removed from a FlFFF elution profile because of factors associated with the accumulation wall membrane.

SEC has been criticized for its lack of consistency in the separation of charged polymers. Part of the problem with SEC is attributed to interactions with the packing material. These interactions are often referred to as 'nonexclusion effects'. Electrolytes can be used to minimize such effects, but the conditions required to avoid both adsorption and repulsion are rather specific to each polymer, and are typically found through trial and error. Verification that an SEC separation is dominated by differences in D or M rather than interactions with the packing material can be a time-consuming process, and still not guarantee the accurate analysis of nonstandard samples. In FFF, the surfaces available for interactions with the sample are greatly reduced by the absence of packing material. Interactions with the accumulation wall can still be a factor, however, since samples are compressed against the wall by the applied field. However, they are less of a problem in FlFFF compared to SEC, and this allows for a wider range of aqueous solutions to be used in the analysis of charged polymers.

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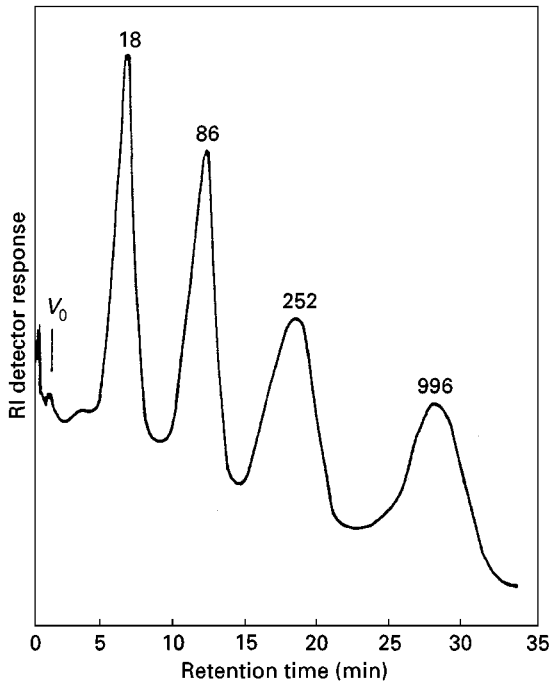

Figure 7 Separation of poly(ethylene oxide) standards by FlFFF. The cross-flow field was programmed to exponentially decay (decay-time constant 8 min) from an initial value of 5.9 mL min^{-1}. Values above peaks are molecular weights expressed as $\times 10^3$ g mol^{-1}. Reprinted with permission from Kirkland JJ, Dilks CH Jr and Rementer SW (1992) Molecular weight distribution of water-soluble polymers by flow field-flow fractionation. *Analytical Chemistry* 64: 1295–1303. Copyright © 1992 American Chemical Society.

FlFFF has been applied to a wide variety of hydrophilic polymers. **Figure 7** illustrates the separation of poly(ethylene oxide) standards with an asymmetrical FlFFF channel using a programmed field. By decaying the field over time, four components ranging in M from 18 000 to 996 000 g mol^{-1} were resolved in 30 min.

Combined with a multi-angle light scattering (MALS) detector, FlFFF is being used to study the conformational dynamics of hydrophilic polymers in solution. Besides its ability to work within a wider range of solvent conditions, its broad size range is responsible for the unique ability of FlFFF–MALS to characterize the structural properties of such polymers in a partially aggregated state.

FlFFF is also being used to study copolymers. For example, the viscometric and aggregation properties of hydrophilic graft copolymers have been studied, as well as the micelle-forming behaviour of such copolymers. FlFFF has also been used to characterize the size and molecular weight of humic and fulvic acids, as well as to study changes in their conformation and aggregation properties as they occur over time upon alterations in solution properties.

Polysaccharides are another class of polymers that have proven difficult to separate by SEC. These materials have a wide range of industrial applications, from coating and packaging to plasma additives and blood substitutes. The physical, biological, and clinical properties of these materials vary with their molecular-weight distribution, which is generally quite broad. It is difficult to prepare robust SEC packings that are capable of analysing these fragile macromolecules without complications of sample adsorption, shear degradation and clogging of the column. FFF has been used to fractionate a wide variety of polysaccharides according to their molecular weight.

FlFFF is used to separate ultra-high molecular-weight polymers, as well as aggregates of lower molecular-weight polymers. For example, SEC fails to completely separate many dextran samples because of the exclusion boundary. The size and molecular weight of such samples are routinely characterized by FlFFF with MALS detection.

Determination of Molecular-Weight Distributions by FFF

The simplest calibration plots in thermal and flow FFF take the following form:

$$\log (V_r) = A + S_m \log M \qquad [9]$$

where A and S_m are calibration constants for a given polymer–solvent system. Parameter S_m is termed the mass-based selectivity. However, at low levels of retention ($R > 0.2$), S_m changes with R. An alternate form of eqn [9] allows for the use of low levels of retention without losing linearity in the calibration plot:

$$\log (V_r - V^\circ) = A + S_m \log M \qquad [10]$$

Equation [10] allows retention to be calibrated over a wide range in molecular weight for a given polymer–solvent system without requiring the calculation of retention parameter λ. The problem remains, however, that neither eqn [9] nor eqn [10] allows for an adjustment in field strength, which is one of the great benefits of FFF, as it allows the field to be optimized for each individual sample. In order to have as single calibration equation for different field strengths, one must incorporate the field strength S:

$$\log \lambda S = B + b \log M \qquad [11]$$

The field strength S is the magnitude of the temperature drop (ΔT) across the channel in ThFFF, and the

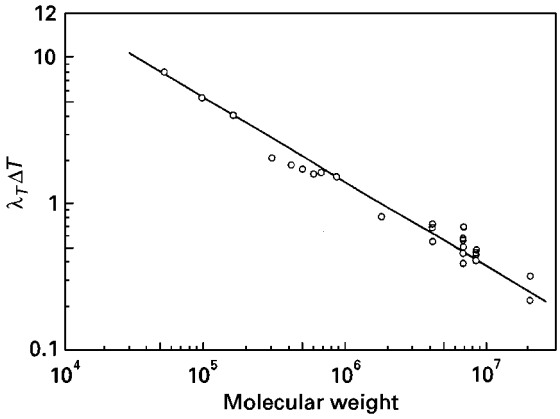

Figure 8 Plot of log $\lambda_T \Delta T$ versus log M for polystyrene samples, illustrating the validity of the calibration model expressed by eqn [11]. The cold wall temperature was 15°C and ΔT ranged from 8 to 81°C. The carrier liquids included tetrahydrofuran and ethylbenzene. A single plot can be used for both solvents because they yield a similar dependence of D/D_T on M for polystyrene. Reprinted with permission from Gao YS, Caldwell KD, Myers MN and Giddings JC (1985) Extension of thermal field–flow fractionation of ultra-high molecular weight polystyrenes. *Macromolecules* 18: 1272. Copyright © 1985 American Chemical Society.

rate of cross flow (V_C) in FlFFF. By calibrating FFF channels in terms of log λS versus log M, the field strength can be changed to optimize the separation of a given sample without the need for re-calibration. **Figure 8** illustrates calibration plots in the form of eqn [11] for ThFFF.

Compared to SEC, universal calibration equations in FFF have the potential for being much broader in scope. In SEC, a given column can be 'universally' calibrated and applied to several types of polymers of similar conformation, provided the intrinsic viscosity is also measured on all standards and samples. In FlFFF, the same concept can be applied, but D is measured directly, so that calibration is not required at all if viscosity is measured independently. In ThFFF, each polymer–solvent system requires a set of different calibration constants, but once such constants are determined, they are applicable to all ThFFF channels in the 'universe'. Of course, the temperature can affect calibration constants, as it does in SEC. Therefore, in ThFFF, the cold wall temperature must be the same in all channels that use a given set of calibration constants.

Trends

FFF will continue to be utilized primarily for the characterization of ultra-high molecular-weight polymers, which are difficult to characterize by SEC. One of the fastest-growing areas for FlFFF is the study of hydrophilic systems that undergo complex interactions. Such interactions are often the key to understanding biological activity in protein and nucleic acid complexes, as well as the complex rheological behaviour of polysaccharides. Regarding the application of ThFFF to industrial polymers, two applications will continue to expand. The first of these is the application to copolymers. As our understanding of thermal diffusion increases, the ability to extract compositional information from fractionated copolymer samples will grow. A growing number of scientists are researching this promising aspect of ThFFF technology.

The second area of growth is the application of ThFFF to the separation of colloidal materials. While this type of sample has been historically considered the domain of flow and sedimentation FFF, the unique ability of ThFFF to separate these materials by composition in both organic and aqueous carrier liquids is gaining the attention of several groups in both industry and academia.

The characterization of polymers will continue to benefit from the combination of FFF with informative detectors such as MALS, dynamic light scattering, intrinsic viscosity, and infrared detectors. For the last thirty years, the characterization of materials by FFF has relied on calibration with standards or the use of retention theory to extract analytical information. Calibration is limited by the availability of polymer standards, and while FFF has the unique ability to produce physicochemical parameters directly from retention theory (i.e. without calibration), this too has limitations. Absolute molecular-weight detectors produce molecular-weight values without the need for calibration curves. When a complex sample is first separated by FFF, a light-scattering detector produces a molecular weight value for hundreds or even thousands of relatively monodisperse components of the sample. The result is a highly accurate determination of the entire molecular-weight distribution of the sample. The combination of FFF–MALS has been particularly popular, as evidenced by the fact that greater than 20% of the papers presented at a recent FFF symposium involved MALS detection.

See also: **II/Chromatograhy: Liquid:** Mechanisms: Size Exclusion Chromatography. **Particle Size Separation:** Theory and Instrumentation of Field Flow Fractionation. Field Flow Fractionation: Thermal.

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Supercritical Fluid Extraction

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Introduction

Plastics are a mixture of the polymer itself and many small molecules. Some, such as antioxidants and plasticizers, are added to the polymer to alter the properties. Others, such as residual monomers, processing aids and feedstock contamination are present inadvertently. The levels of these compounds must be accurately known by manufacturers and regulators in order to assess whether the plastic is fit for its intended purpose. There are usually many compounds present in the plastic, which makes analysis of their levels whilst still in the plastic very difficult. Usually, therefore, the compounds must be separated from the bulk polymer before analysis. Conventional methods include liquid/solid extraction and dissolution followed by reprecipitation of the polymer. Conventional solvent extraction methods tend to be very slow, e.g. Soxhlet extraction may require 24 hours to complete, and the dissolution/reprecipitation methods may result in extracts contaminated by oligomeric 'waxes', requiring further clean up before analysis. Methods producing clean, fast extracts are therefore very useful. The techniques of supercritical fluid extraction (SFE), pressurized fluid extraction (PFE) and microwave assisted extraction (MAE) have been shown to decrease extraction times, with lower use of solvents than conventional methods.

The Extraction Process

In SFE and PFE, the matrix is held in a cell, and the solvent is pumped into the cell under pressure. Commonly in SFE, the solvent is pumped continuously past the sample (dynamic extraction), dissolving the analyte molecule and carrying it out of the cell to be collected. In PFE it is more common for the solvent to be pumped until the cell is full, and then left for a period of static extraction. The analyte dissolves in the solvent, which is then flushed by more solvent from the cell to the collecting vial. MAE is carried out in one container, in which sample and solvent are placed. The solvent is heated by microwaves, and the analytes dissolve in the solvent. The vessel must then be allowed to cool before opening, and the extraction liquid can be separated from the extracted polymer by simple filtering. In simple terms, extractions with all methods can be thought of as proceeding in two